

SEQUENCE LISTING

Pub D
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Yabuta, Masayuki
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<120> Process for Producing Peptides Using a Helper Peptide

<130> 001560-373

<140> US 09/402,093

<141> 1999-09-29

<150> PCT/JP99/00406

<151> 1999-01-29

<150> JP 10-32272

<151> 1998-01-30

<160> 24

<170> PatentIn version 3.0

<210> 1

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by enterokinase.

<400> 1

Asp Asp Asp Lys

1

B1
<210> 2

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence adjacent to a site cleaved by blood
coagulation Factor Xa.

<400> 2

Ile Glu Gly Arg

1

<210> 3

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

Sub-D1
 <223> Amino acid sequence containing a site cleaved by renin.

<400> 3

Pro Phe His Leu Leu Val Tyr
 1 5

<210> 4

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 4

Val Asp Asp Asp Asp Lys
 1 5

<210> 5

<211> 6

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 5

Gly Cys His His His His
 1 5

<210> 6

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a chemically cleaved site.

<400> 6

Pro Gly Gly Arg Pro Ser Arg His Lys Arg
 1 5 10

<210> 7

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of helper peptide.

<400> 7

His Arg His Lys Arg Ser His His His His

1 5 10

<210> 8
 <211> 5
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Amino acid sequence containing a site cleaved by Kex2 protease.

<400> 8

Ser Asp His Lys Arg
 1 5

<210> 9
 <211> 23
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Amino acid sequence containing a position cleaved by OmpT.

<400> 9

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
 1 5 10 15

Arg Trp Gly Arg Ser Gly Ser
 20

<210> 10
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Amino acid sequence containing a position cleaved by OmpT.

<400> 10

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
 1 5 10 15

Gly Ser Gly Ser
 20

<210> 11
 <211> 69
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for an amino acid sequence containing
 a site cleaved by OmpT

<220>

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 <222> (1)..(69)

<400> 11

cag atg cat ggt tat gac gcg gag ctc cgg ctg tat cgc cgt cat cac 48
 Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
 1 5 10 15

cgg tgg ggt cgt tcc gga tcc 69
 Arg Trp Gly Arg Ser Gly Ser
 20

<210> 12

<211> 23

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by OmpT.

<400> 12

Gln Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His
 1 5 10 15

Arg Trp Gly Arg Ser Gly Ser
 20

<210> 13

<211> 47

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for an amino acid sequence containing
 a site cleaved by OmpT

<400> 13

tggttatgac gcggagctcc gcctgtatcg ccgtcatcac gggtccg 47

<210> 14

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> Nucleotide sequence coding for an amino acid sequence containing
 a site cleaved by OmpT

<400> 14

gatccggaac cgtgatgacg gcgatacagg cggagctccg cgtcataacc atgca 55

<210> 15
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 15
 gactcagatc ttccctgaggc cgat

24

<210> 16
 <211> 36
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 16
 aaaggtacct tccgcatgcc gcgatgtcg agaagg

36

<210> 17
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 17
 aggccaggaa ccgtaaaaag

20

<210> 18
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Primer.

<400> 18
 aaaatgcac gcacgcgtaac cgtgcacatc

29

<210> 19
 <211> 627
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Nucleotide sequence coding for a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<220>
 <221> CDS
 <222> (82)..(543)

<400> 19

cccaggcttt acacttttatg cttccggctc gtatgttggtg tggaattgtg agcggataac 60

aatttcacac aggaaacagc t atg acc atg att acg gat tca ctg gcc gtc 111
 Met Thr Met Ile Thr Asp Ser Leu Ala Val
 1 5 10

gtt tta caa cgt aaa gac tgg gat aac cct gcc gtt acc caa ctt aat 159
 Val Leu Gln Arg Lys Asp Trp Asp Asn Pro Gly Val Thr Gln Leu Asn
 15 20 25

cgc ctt gca gca cat ccc cct ttc gcc agc tgg cgt aat agc gac gac 207
 Arg Leu Ala Ala His Pro Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp
 30 35 40

gcc cgc acc gat cgc cct tcc caa cag ttg cgc agc ctg aat gcc gaa 255
 Ala Arg Thr Asp Arg Pro Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu
 45 50 55

tgg cgc ttt gcc tgg ttt ccg gca cca gaa gcg gtg ccg gca agc ttg 303
 Trp Arg Phe Ala Trp Phe Pro Ala Pro Glu Ala Val Pro Ala Ser Leu
 60 65 70

ctg gag tca gat ctt cct gac gcc gat act gtc gtc gtc ccc tca aac 351
 Leu Glu Ser Asp Leu Pro Asp Ala Asp Thr Val Val Val Pro Ser Asn
 75 80 85 90

tgg cag atg cac ggt tac gat gcg atg cat ggt tat gac gcg gag ctc 399
 Trp Gln Met His Gly Tyr Asp Ala Met His Gly Tyr Asp Ala Glu Leu
 95 100 105

cgc ctg tat cgc cgt cat cac ggt tcc gga tcc cct tct cga cat ccg 447
 Arg Leu Tyr Arg Arg His His Gly Ser Gly Ser Pro Ser Arg His Pro
 110 115 120

cgg cat gcg gaa ggt acc ttt acc agc gat gtg agc tcg tat ctg gaa 495
 Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu
 125 130 135

ggt cag gcg gca aaa gaa ttc atc gcg tgg ctg gtg aaa gcc cgt ggt 543
 Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 140 145 150

taagtcgaca gcccgccctaa tgagcgggct tttttttctc ggaattaatt ctcatgtttg 603

acagcttatc atcgataagc tttta 627

<210> 20
 <211> 154
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 20

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80

Asp Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95

Asp Ala Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His
 100 105 110

His Gly Ser Gly Ser Pro Ser Arg His Pro Arg His Ala Glu Gly Thr
 115 120 125

Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu
 130 135 140

Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 145 150

<210> 21

<211> 187

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 21

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15
 Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
 50 55 60
 Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
 65 70 75 80
 Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
 85 90 95
 Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
 100 105 110
 Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln
 115 120 125
 Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Arg
 130 135 140
 Trp Gly Arg Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly
 145 150 155 160
 Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys
 165 170 175
 Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly
 180 185

<210> 22
 <211> 184
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 22

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
 1 5 10 15
 Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
 20 25 30
 Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
 35 40 45
 Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe

50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
65 70 75 80

Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
100 105 110

Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln
115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly
130 135 140

Ser Gly Ser Pro Ser Arg His Lys Arg His Ala Glu Gly Thr Phe Thr
145 150 155 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile
165 170 175

Ala Trp Leu Val Lys Gly Arg Gly
180

<210> 23

<211> 184

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence of a fusion protein comprising GLP-1, helper peptide and beta-galactosidase protective peptide.

<400> 23

Met Thr Met Ile Thr Asp Ser Leu Ala Val Val Leu Gln Arg Lys Asp
1 5 10 15

Trp Asp Asn Pro Gly Val Thr Gln Leu Asn Arg Leu Ala Ala His Pro
20 25 30

Pro Phe Ala Ser Trp Arg Asn Ser Asp Asp Ala Arg Thr Asp Arg Pro
35 40 45

Ser Gln Gln Leu Arg Ser Leu Asn Gly Glu Trp Arg Phe Ala Trp Phe
50 55 60

Pro Ala Pro Glu Ala Val Pro Ala Ser Leu Leu Glu Ser Asp Leu Pro
65 70 75 80

Glu Ala Asp Thr Val Val Val Pro Ser Asn Trp Gln Met His Gly Tyr
85 90 95

Asp Ala Pro Ile Tyr Thr Asn Val Thr Tyr Pro Ile Thr Val Asn Pro
100 105 110

Pro Phe Val Pro Thr Glu Pro His His His His His Gly Gly Arg Gln
 115 120 125

Met His Gly Tyr Asp Ala Glu Leu Arg Leu Tyr Arg Arg His His Gly
 130 135 140

Ser Gly Ser Pro Ser Arg His Pro Arg His Ala Glu Gly Thr Phe Thr
 145 150 155 160

Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile
 165 170 175

Ala Trp Leu Val Lys Gly Arg Gly
 180

<210> 24

<211> 5

<212> PRT

<213> Artificial Sequence

<220>

<223> Amino acid sequence containing a site cleaved by Kex2 Protease.

<400> 24

Ser Cys His Lys Arg
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